Title: **PHOTOTROPHIC CARBON ASSIMILATION ASSAY**

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1.0 OBJECTIVE

To measure the rate of assimilation of radiolabeled carbon by phytoplankton as an estimate of phototrophic productivity, as described in Parsons, *et al*. (1984).

2.0 HEALTH AND SAFETY

This procedure uses radioactive materials and may only be performed in a certified radiation area. Appropriate caution must be taken to minimize exposure and contamination (see training required, section 3.0). Personal protection equipment including lab coat, film badge and chemical resistant gloves will be worn at all times. Work will be performed under a fume hood when possible.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

This procedure requires radiation safety training and certification. Only those individuals having completed training through the Radiation Safety Officer and possessing a current film badge are permitted to perform the procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

$NaH^{14}CO_3$
0.45 µm cellulose nitrate membrane filters
temperature and light controlled incubator
7 mL plastic scintillation vials with screw caps
10% HCl
scintillation fluid
liquid scintillation counter
pipettes
filter forceps
filter apparatus and vacuum pump

5.0 PROCEDURE

5.1 Analysis

- Spike each 5 mL water sample with 1 µCi/ml $NaH^{14}CO_3$ (final concentration). This sample volume and isotope concentration is adequate for most estuarine PFU samples. It is necessary; however, to first determine the optimal isotope concentration for each study, as phototrophic uptake varies with season and location.
- Replicate samples (to estimate dark uptake) should be spiked with the same $NaH^{14}CO_3$ concentration and then immediately covered with aluminum foil.
- Place samples in an incubator set at *in situ* light and temperature regimes.
- After 24 hours, filter samples through 0.45 µm cellulose nitrate membrane filters then rinse with 0.2 µm filtered seawater.
- Place filters in scintillation vials and add 1 mL of 10% HCl (v/v).
- Allow samples to fume overnight in the dark.
- The next day, add 5 mL of scintillation fluid to each vial.
- After a 24-h incubation (stabilization) period, measure radioactivity as disintegrations per minute (DPM) using a liquid scintillation counter. Again, the stabilization period is an estimate and should be optimized for each study. As scintillation counters vary considerably, please see laboratory supervisor for assistance with programming and set-up.

5.2 Calculations

- Phototrophic carbon assimilation is calculated as follows:

  $$\text{mg C/L/d} = 1.05 \times (Y/Z) \times W$$

  where,
  1.05 is a correction factor for isotopic discrimination
  Y is the CPM of the sample corrected for dark uptake
  Z is the CPM of the $NaH^{14}CO_3$ stock solution
  W is the total carbonate content of the water sample

6.0 QUALITY CONTROL/QUALITY ASSURANCE

Samples should always be corrected for dark uptake. The samples are acidified and fumed to drive off inorganic carbon. The scintillation counter should be set to minimize interferences such as quenching. These controls help avoid overestimation of phototrophic uptake. A minimum of three replicates per site or treatment is recommended.

7.0 REFERENCES

Parsons, T.R., Y. Maita, and C.M. Lalli. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford.